In vivo expression and analysis of a novel protein, NIAM, in *Drosophila melanogaster*

Katie Thies[1], Jussara Hagen[2], Diane E. Cryderman[3], Lori L. Wallrath[3] and Dawn E. Quelle[2]
Biology Undergraduate Program[1], Department of Pharmacology[2], Department of Biochemistry[3]

Background & Significance:

• The novel protein known as NIAM (Nuclear Interactor of ARF and MDM2) functions to inhibit proliferation, bind chromatin *in vitro* and maintain chromosomal stability.[1] NIAM contains FYRN/FYRC domains enriched in phenylalanine and tyrosine residues (50-100aa).[2]

• Many chromatin-associated proteins, particularly histone H3K4 methyltransferases, contain these FYRN/FYRC motifs.[2] However, the function of these unique domains is not known.

• Drosophila NIAM (dNIAM) also retains FYRN/FYRC domains, which we predict associate with chromatin similarly to the mammalian form.

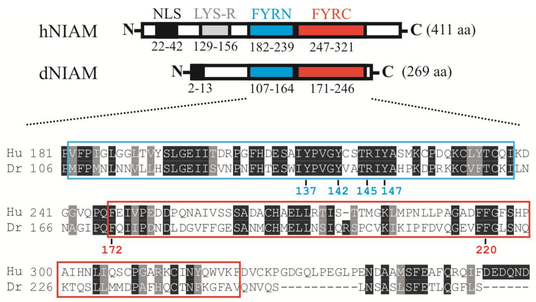


Fig 1. Comparison of human and Drosophila NIAM. Schematics highlight the location of nuclear localization (NLS), lysine-rich (LYS-R) and FYRN/FYRC domains in each protein (top). Sequence comparison of the FYRN (blue box) and FYRC (red box) regions is shown with identical aa shaded in black, similar residues in gray. Conserved residues predicted to play a key role in folding and structure of the FYRN/FYRC domains, based on structural studies of hNIAM (4), are highlighted.

Overall Research Goals:

• Test if NIAM is a chromatin associated protein *in vivo*

• Determine the biological role of dNIAM, a novel protein, in *Drosophila melanogaster*

References:

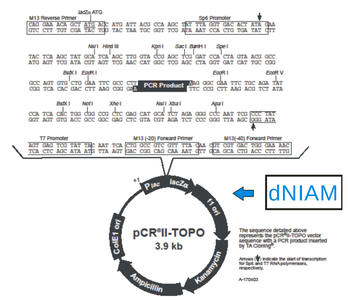
1.Tompkins VS, Hagen J, Frazier AA, et al. A Novel Nuclear Interactor of ARF and MDM2 (NIAM) That Maintains Chromosomal Stability. Journal of Biological Chemistry 2007; 282:1322-33.
2.Garcia-Alai MM, Allen MD, Joerger AC, Bycroft M. The structure of the FYR domain of transforming growth factor beta regulator 1. Protein Science; 19:1432-8.

Acknowledgments:
Thank you to Sara Francis, my student research mentor, for encouragement and guidance.

Methods:

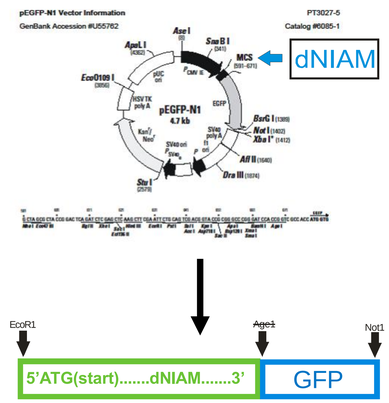
A) PCR amplified *dNIAM* cDNA using primers with 5' EcoR1 and 3' Age1
- Added A overhangs by Taq polymerase

B) Subcloned into pCRII-TOPO vector

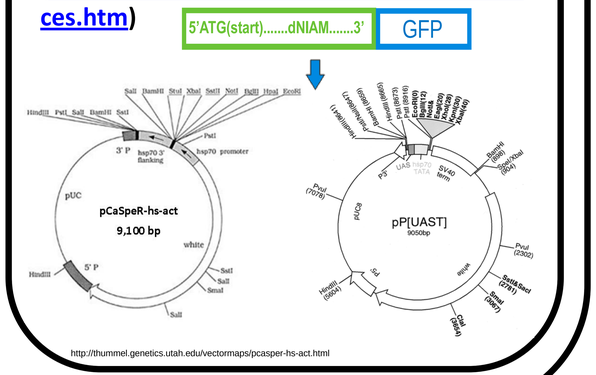


- sequenced miniprep DNA
- digested recombinant plasmid to release the *dNIAM* insert

C) Cloned *dNIAM* cDNA into pEGFP vectors in order to form fusion constructs with GFP (*dNIAM-GFP*)



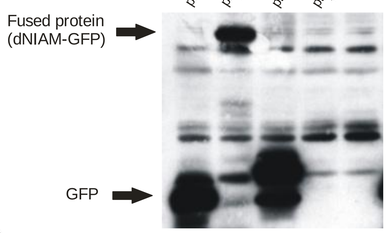
D) Digested and ligated *dNIAM-GFP* cDNA into *Drosophila melanogaster* GAL4-UAS inducible expression vectors, which were subsequently injected into *Drosphila* larvae by a company to obtain *in vivo* expression (http://www.geneticservices/injectionservices.htm)



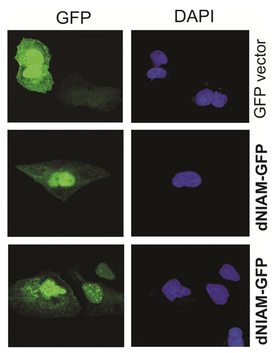
Results: dNIAM-GFP is nuclear in human cells

(1) Western blotting confirmed appropriate size of dNIAM-GFP fusion protein when expressed in 293T mammalian cells
(2) Immmunfluorescence analysis in same cells confirmed fusion protein localization in nucleus, as expected

Western Blot:



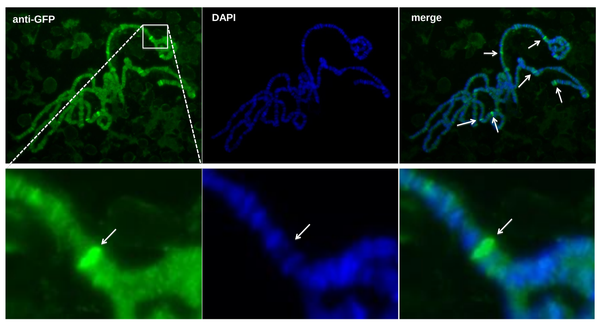
Immunofluorescence Image:



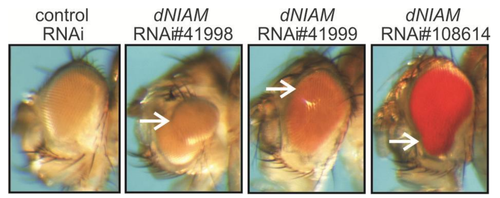
Future Directions:

• Construct mutant forms of dNIAM that lack FYRN/FYRC motifs and express in *Drosophila*
• Determine if FYRN/FYRC domains in dNIAM interact with chromatin modification machinery
• Determine if the FYRN/FYRC domains of NIAM are critical for chromatin association, chromosome stability and/or proliferation

Results: dNIAM-GFP binds chromatin *in vivo* when expressed in flies



Transgenic fly stocks (from D) were generated and crossed to get dNIAM-GFP expression. Salivary glands were fixed, squashed and stained with antibodies to GFP. **dNIAM-GFP localized to chromosomes *in vivo* at decondensed regions of chromatin (no DAPI staining), consistent with a role in chromatin regulation.**



Other studies showed that **loss of endogenous *dNIAM* by RNAi causes an over-proliferation phenotype in eyes**